

WHAT IS CLAIMED IS:

1. A method of establishing a feeder cells-free human embryonic stem cell line capable of being maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:
 - (a) obtaining human embryonic stem cells, and;
 - (b) culturing said human embryonic stem cells under culturing conditions devoid of feeder cells and including a matrix and a tissue culture medium supplemented with TGF β ₁, bFGF and/or LIF to thereby obtain the feeder cells-free human embryonic stem cell line.
2. The method of claim 1, further comprising cloning a cell from the human embryonic stem cell line resultant from step (b) under said culturing conditions.
3. The method of claim 1, wherein said matrix is a fibronectin matrix.
4. The method of claim 3, wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin.
5. The method of claim 1, wherein said culturing conditions are substantially free of xeno contaminant and whereas said matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix, synthetic fibronectin.
6. The method of claim 1, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.
7. The method of claim 1, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.

8. The method of claim 1, wherein said tissue culture medium further includes serum and/or serum replacement.

9. The method of claim 8, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

10. The method of claim 8, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

11. The method of claim 1, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

12. The method of claim 1, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

13. The method of claim 1, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

14. The method of claim 1, wherein said bFGF is provided at a concentration of 4 ng/ml.

15. The method of claim 1, wherein said LIF is provided at a concentration of at least 500 u/ml.

16. The method of claim 1, wherein said LIF is provided at a concentration of 1000 u/ml.

17. A method of propagating a human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing cells of the human embryonic stem cell line on a matrix and a tissue culture medium supplemented with $TGF\beta_1$, bFGF

and/or LIF to thereby maintain the cells of the human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

18. The method of claim 17, wherein said matrix is a fibronectin matrix.

19. The method of claim 18, wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin.

20. The method of claim 17, wherein said culturing conditions are substantially free of xeno contaminant and where is said matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix, synthetic fibronectin.

21. The method of claim 17, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.

22. The method of claim 17, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.

23. The method of claim 17, wherein said tissue culture medium further includes serum and/or serum replacement.

24. The method of claim 23, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

25. The method of claim 23, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

26. The method of claim 17, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

27. The method of claim 17, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

28. The method of claim 17, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

29. The method of claim 17, wherein said bFGF is provided at a concentration of 4 ng/ml.

30. The method of claim 17, wherein said LIF is provided at a concentration of at least 500 u/ml.

31. The method of claim 17, wherein said LIF is provided at a concentration of 1000 u/ml.

32. A method of establishing a feeder cells-free human embryonic stem cell line capable of being maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

- (a) obtaining human embryonic stem cells, and;
- (b) culturing said human embryonic stem cells under culturing conditions devoid of feeder cells and including a fibronectin matrix and a tissue culture medium supplemented with $\text{TGF}\beta_1$, bFGF and/or LIF to thereby obtain the feeder cells-free human embryonic stem cell line.

33. The method of claim 32, wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin.

34. The method of claim 32, wherein said culturing conditions are substantially free of xeno contaminant and where said fibronectin matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human

plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix and synthetic fibronectin.

35. The method of claim 32, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.

36. The method of claim 32, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.

37. The method of claim 32, wherein said tissue culture medium further includes serum and/or serum replacement.

38. The method of claim 37, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

39. The method of claim 37, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

40. The method of claim 32, wherein said $\text{TGF}\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

41. The method of claim 32, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

42. The method of claim 32, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

43. The method of claim 32, wherein said bFGF is provided at a concentration of 4 ng/ml.

44. The method of claim 32, wherein said LIF is provided at a concentration of at least 500 u/ml.

45. The method of claim 32, wherein said LIF is provided at a concentration of 1000 u/ml.
46. A method of propagating a human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing cells of the human embryonic stem cell line on a fibronectin matrix and a tissue culture medium supplemented with TGF β ₁, bFGF and/or LIF to thereby maintain the cells of the human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.
47. The method of claim 46, wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin.
48. The method of claim 46, wherein said culturing conditions are substantially free of xeno contaminant and where said fibronectin matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix, and synthetic fibronectin.
49. The method of claim 46, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.
50. The method of claim 46, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.
51. The method of claim 46, wherein said tissue culture medium further includes serum and/or serum replacement.
52. The method of claim 51, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

53. The method of claim 51, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

54. The method of claim 46, wherein said $\text{TGF}\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

55. The method of claim 46, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

56. The method of claim 46, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

57. The method of claim 46, wherein said bFGF is provided at a concentration of 4 ng/ml.

58. The method of claim 46, wherein said LIF is provided at a concentration of at least 500 u/ml.

59. The method of claim 46, wherein said LIF is provided at a concentration of 1000 u/ml.

60. A method of establishing a xeno – free, feeder cells-free embryonic stem cell line of a species capable of being maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

- (a) obtaining embryonic stem cells, and;
- (b) culturing said embryonic stem cells under culturing conditions devoid of feeder cells and xeno contaminants and including a species - derived matrix and a tissue culture medium to thereby obtain the xeno – free, feeder cells-free embryonic stem cell line of the species.

61. The method of claim 60, wherein said matrix is a species – derived fibronectin matrix.

62. The method of claim 60, wherein said feeder cells-free culturing conditions are substantially free of xeno contaminants.

63. The method of claim 60, wherein the species embryonic stem cell line comprises at least 85 % of undifferentiated species embryonic stem cells.

64. The method of claim 60, wherein the cells of the species embryonic stem cell line maintain a doubling time of at least 20 hours.

65. The method of claim 60, wherein said tissue culture medium includes a species - derived serum and/or a serum replacement.

66. The method of claim 65, wherein said species - derived serum is provided at a concentration of at least 5 %.

67. The method of claim 65, wherein said serum replacement is provided at a concentration of at least 10 %.

68. The method of claim 65, wherein said serum replacement is provided at a concentration of 15 %.

69. The method of claim 60, wherein said tissue culture medium further includes at least one growth factor.

70. The method of claim 69, wherein said at least one growth factor is selected from the group consisting of $TGF\beta_1$, bFGF, LIF.

71. The method of claim 70, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

72. The method of claim 70, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

73. The method of claim 70, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

74. The method claim 70, wherein said bFGF is provided at a concentration of 4 ng/ml.

75. The method of claim 70, wherein said LIF is provided at a concentration of at least 500 u/ml.

76. The method of claim 70, wherein said LIF is provided at a concentration of 1000 u/ml.

77. The method of claim 60, wherein said tissue culture medium is a species – derived conditioned medium.

78. A method of propagating a species embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells and xeno contaminants, the method comprising culturing cells of the species embryonic stem cell line on a species - derived matrix and a tissue culture medium to thereby maintain the cells of the species embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

79. The method of claim 78, wherein said matrix is a species – derived fibronectin matrix.

80. The method of claim 78, wherein said feeder cells-free culturing conditions are substantially free of xeno contaminants.

81. The method of claim 78, wherein the species embryonic stem cell line comprises at least 85 % of undifferentiated species embryonic stem cells.

82. The method of claim 78, wherein the cells of the species embryonic stem cell line maintain a doubling time of at least 20 hours.

83. The method of claim 78, wherein said tissue culture medium includes a species - derived serum and/or a serum replacement.

84. The method of claim 83, wherein said species - derived serum is provided at a concentration of at least 5 %.

85. The method of claim 83, wherein said serum replacement is provided at a concentration of at least 10 %.

86. The method of claim 83, wherein said serum replacement is provided at a concentration of 15 %.

87. The method of claim 83, wherein said tissue culture medium further includes at least one growth factor.

88. The method of claim 87, wherein said at least one growth factor is selected from the group consisting of $\text{TGF}\beta_1$, bFGF, and LIF.

89. The method of claim 88, wherein said $\text{TGF}\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

90. The method of claim 88, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

91. The method of claim 88, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

92. The method claim 88, wherein said bFGF is provided at a concentration of 4 ng/ml.

93. The method of claim 88, wherein said LIF is provided at a concentration of at least 500 u/ml.

94. The method of claim 88, wherein said LIF is provided at a concentration of 1000 u/ml.

95. The method of any of claim 78, wherein said tissue culture medium is a species – derived conditioned medium.

96. A cell culture comprising undifferentiated, pluripotent and proliferative human embryonic stem cells in a culture medium, wherein the cell culture is substantially free of xeno- and/or feeder cells contaminants.

97. The cell culture of claim 96, wherein the culture medium includes serum replacement.

98. The cell culture of claim 97, wherein said serum replacement is provided at a concentration of at least 10 %.

99. The cell culture of claim 97, wherein said serum replacement is provided at a concentration of 15 %.

100. The cell culture of claim 97, wherein said culture medium further includes $TGF\beta_1$, bFGF and/or LIF.

101. The cell culture of claim 100, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

102. The cell culture of claim 100, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

103. The cell culture of claim 100, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

104. The cell culture of claim 100, wherein said bFGF is provided at a concentration of 4 ng/ml.

105. The cell culture of claim 100, wherein said LIF is provided at a concentration of at least 500 u/ml.

106. The cell culture of claim 100, wherein said LIF is provided at a concentration of 1000 u/ml.

107. The cell culture of claim 96, wherein said human embryonic stem cells are maintainable in an undifferentiated, pluripotent and proliferative state for at least 38 passages.

108. The cell culture of claim 96, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

109. The cell culture of claim 96, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated stem cells.

110. A xeno-free, feeder cells-free culture system comprising a matrix and a tissue culture medium, the xeno-free, feeder cells-free culture system being selected capable of maintaining human embryonic stem cells cultured therein in a proliferative, pluripotent and undifferentiated state.

111. The culture system of claim 110, wherein said matrix is human-derived fibronectin.

112. The culture system of claim 111, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

113. The culture system of claim 110, wherein said tissue culture medium includes serum replacement.

114. The culture system of claim 113, wherein said serum replacement is provided at a concentration of at least 10 %.

115. The culture system of claim 113, wherein said serum replacement is provided at a concentration of 15 %.

116. The culture system of claim 110, wherein said tissue culture medium further includes $\text{TGF}\beta_1$, bFGF and/or LIF.

117. The culture system of claim 116, wherein said $\text{TGF}\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

118. The culture system of claim 116, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

119. The culture system of claim 116, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

120. The culture system of claim 116, wherein said bFGF is provided at a concentration of 4 ng/ml.

121. The culture system of claim 116, wherein said LIF is provided at a concentration of at least 500 u/ml.

122. The culture system of claim 116, wherein said LIF is provided at a concentration of 1000 u/ml.

123. The method of claim 110, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

124. The method of claim 110, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

125. A method of treating an individual in need of cell replacement and/or tissue regeneration, comprising administering a human embryonic stem cell preparation being free of xeno and feeder cells contaminants to the individual.

126. The method of claim 125, further comprising preparing said human embryonic stem cell preparation prior to said administering, said preparing being effected by:

- (a) obtaining human embryonic stem cells, and;
- (b) culturing said human embryonic stem cells under culturing conditions devoid of feeder cells and xeno contaminants and including a human-derived fibronectin matrix and a tissue culture medium supplemented with $TGF\beta_1$, bFGF and/or LIF to thereby prepare the human embryonic stem cell preparation.

127. The method of claim 126, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

128. The method of claim 126, wherein said tissue culture medium includes human serum and/or serum replacement.

129. The method of claim 128, wherein said human serum and/or said serum replacement is provided at a concentration of at least 10 %.

130. The method of claim 128, wherein said human serum and/or said serum replacement is provided at a concentration of 15 %.

131. The method of claim 126, wherein said $\text{TGF}\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

132. The method of claim 126, wherein said $\text{TGF}\beta_1$ is provided at a concentration of 0.12 ng/ml.

133. The method of claim 126, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

134. The method of claim 126, wherein said bFGF is provided at a concentration of 4 ng/ml.

135. The method of claim 126, wherein said LIF is provided at a concentration of at least 500 u/ml.

136. The method of claim 126, wherein said LIF is provided at a concentration of 1000 u/ml.

137. The method of claim 126, wherein said human embryonic stem cell preparation comprises at least 85 % of undifferentiated human embryonic stem cells.

138. The method of claim 126, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

139. A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human embryonic stem cells under culturing conditions including a matrix and tissue culture medium supplemented with at least one growth factor provided at a concentration range selected capable of maintaining said stem cells for at least 56 passages with a doubling time of at least 25 hours.

140. The method of claim 139, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

141. The method of claim 139, wherein said matrix is selected from the group consisting of human-derived fibronectin, human-derived laminin, foreskin fibroblast matrix, MEFs matrix.

142. The method of claim 141, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

143. The method of claim 139, wherein said culturing conditions include human serum and/or serum replacement.

144. The method of claim 143, wherein said human serum is provided at a concentration of at least 5 %.

145. The method of claim 143, wherein said human serum is provided at a concentration of 10 %.

146. The method of claim 143, wherein said serum replacement is provided at a concentration of at least 10 %.

147. The method of claim 143, wherein said serum replacement is provided at a concentration of 15 %.

148. The method of claim 139, wherein said at least one growth factor is selected from the group consisting of $\text{TGF}\beta_1$, bFGF, and LIF.

149. The method of claim 148, wherein said $\text{TGF}\beta_1$ is provided at a concentration range of 0.06-0.24 ng/ml.

150. The method of claim 148, wherein said bFGF is provided at a concentration range of 2-8 ng/ml.

151. The method of claim 149, wherein said LIF is provided at a concentration range of 500-2000 u/ml.

152. The method of claim 139, wherein said culturing conditions include serum replacement at a concentration of 15 %, $\text{TGF}\beta_1$ at a concentration of 0.12 ng/ml, LIF at a concentration of 1000 u/ml, and bFGF at a concentration of 4 ng/ml.